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Optimization of Madeira Vine Leaves and Aloe vera Extracts in PVA/Gelatin Hydrogel for Antibacterial Wound Dressing

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Abstract

Natural ingredients containing antibacterial substances, such as those found in Aloe vera and Madeira vine (*Anredera cordifolia*) plants, are needed to fasten wound healing and address bacterial infections. The combinations of these plant extracts were formulated into a hydrogel prepared by mixing the extracts with polyvinyl alcohol (PVA) and shaving gelatin waste. The present study was aimed to determine the optimum combination of madeira vine leaves and aloe vera extracts impregnated to PVA/gelatin hydrogel that give the highest antibacterial activity. The plant extracts were prepared by maceration procedure using 96% ethanol as solvent followed by evaporation to obtain concentrated extracts. The extracts were then screened for their phytochemical content. Madeira vine leaves extract contains phenolic, tannins, flavonoids, saponins, and triterpenoids compounds. Meanwhile, aloe vera extract contains flavonoids, alkaloids, and triterpenoids compounds. Antibacterial assay using spectrophotometry method showed 39.13% bacterial growth inhibition when the assay solution contains 1000 ppm madeira vine leaves extract, while no inhibition observed with the aloe vera extract. The highest antibacterial activity in the hydrogel was found when madeira vine leaves and aloe vera extracts were adjusted to 250 and 750 ppm, respectively, giving the lowest cell growth rate (μ). The optimized hydrogel was then characterized using FTIR analysis, swelling ratio, and *in vitro* extract release. FTIR spectrum reveals the presence of -OH stretching, C-H stretching, C=C stretching aromatic, C=O stretching, C-N stretch and N-H bending. The swelling ratio was 19.1%, exceeding the specified standard swelling ratio of 12.5%, while the *in vitro* extract release test shows an extract release percentage of 28.5% within 180 minutes.

Keywords: Madeira vine leaves, Aloe vera, Gelatin, Hydrogel, Wound dressing.

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1. Introduction

Untreated open wounds in animals can lead to infections, potentially spreading to deeper tissues and hindering the healing process [1]. Proper wound care is crucial to prevent complications, and while the animal wound treatment industry has made progress, antibiotics remain a primary component [2]. To fasten wound healing and address bacterial infections, natural ingredients with antibacterial properties, such as aloe vera and madeira vine (known in Indonesia as *binahong*) leaves, are essential. Aloe vera, known for its antibacterial, anti-fungal, anti-inflammatory attributes and has been proven effective in wound healing [3-5]. Madeira vine leaves, an underutilized medicinal plant, meet criteria for antibacterial effectiveness, availability, affordability, minimal side effects, and easy cultivation [6]. Hydrogels, commonly used in wound healing research, prove effective in debris removal, maintaining a moist environment, and providing a cooling effect, ultimately contributing to pain reduction and enhancing patient comfort during recovery [7]. Hydrogel can be prepared by mixing polyvinyl alcohol and gelatin [8-9]. Gelatin can be prepared from leather processing waste and usually contain high amount of chrome. By utilizing this waste, can gives benefit by reducing the amount of waste to the environmental [10].

Combining hydrogel with natural antibacterial extract will lead to a biocompatible material with high effectivity and affordability that can be applied in wound healing. Madeira vine leaves and aloe vera extracts in hydrogel will improve wound healing process, where antibacterial property from madeira vine leaves extract, aloe vera's cell-stimulating effects complement with gelatin's moisture-absorbing properties [11]. Based on the discussion above, the authors consider that it is important to investigate the study entitled "Optimization of Madeira Vine Leaves and Aloe vera Extracts in PVA/Gelatin Hydrogel for Antibacterial Wound Dressing".

2. Materials and methods

2.1. Research Design

The design of the hydrogel film formulation was carried out using Minitab 17 software, for optimization calculations using a 2-level 2 factorial design with 2 replications resulting in 8 experiments. The experiments to be analyzed are shown in Table 1.

2.2. Preparation of Aloe vera and Madeira vine leaves extracts

The preparation of aloe vera gel and madeira vine leaves extracts involve maceration with 1:2 ratio sample to 96% ethanol. After 24 hours, the liquid was filtered, followed by concentrating using vacuum rotary evaporator at 60ºC [12-13]. The result of evaporation gives liquid aloe vera extract and pasta madeira vine leaves extract.

2.3. Phytochemical Screening

Phytochemical tests for phenolic, tannin, flavonoid, alkaloid, saponin, steroid and tripetenoid was performed as described by Mailuhu et al., (2017) [14].

2.4. Antibacterial Assay

Antibacterial assay was performed following method described by Akinduti et al., (2019) with some modifications [15]. Briefly, for assay of extracts, an overnight culture of Staphylococcus aureus ATCC 25923 was transferred to four sterilized Erlenmeyer flask each containing 15 mL Mueller Hinton Broth (MHB) media to gives initial OD value of 0.1. A 96% ethanol, gentamicin, madeira vine leaves extract, and aloe vera extract was added to each Erlenmeyer flask, respectively to gives final concentration of 1000 ppm. The culture was shaken at 180 rpm. The OD value at 600 nm was recorded every 2 hours for 8 hours. The bacterial growth rate was calculated based on the slope of the OD to time graph. For antibacterial assay of hydrogel, the same protocol was conducted with substitution of extracts solution with hydrogel film (ø 3.8 cm).

2.5. Preparation of Hydrogel Film

The hydrogels were prepared by heating 20 g of PVA in 200 mL water with 400 rpm stirring speed until clear solution was obtained. Afterward, 5 g of gelatin and 0.1 mL of 37% HCl was added to the solution. The mixture was then stirred (400 rpm) at 75°C for 30 minutes. The solution was divided to 10 equal volumes, eight of which were added by the combination of the extracts as presented on Table 1. One was added by gentamicin to gives 1000 ppm final concentration, while the last one was leave blank without any addition. The mixture was stirred manually followed by sonication for 20 minutes and casting on petri dish. The samples were then dried in an oven at 40°C for 48 hours [8].

2.6. FTIR (Fourier Transform Infrared) Analysis

Hydrogel films were prepared by grounded and mixed at a ratio of 1.0% with potassium bromide (KBr) powder which was dried for 24 hours at 120°C. FTIR spectra were collected in the range of 4000-400 cm^{-1}, with a scan rate of 2 mm/sec and a resolution of 4 cm^{-1} [8].

2.7. Swelling Ratio

The swelling ratio assay analyzes the hydrogel's ability to expand or absorb water. The water absorption capacity of the hydrogel film was determined as suggested by Lim et al., (2015) with some modification by immersing the hydrogel film (ø 3.8 cm) in 8 mL distilled water. Then the diameter was measured after 5, 15, 25, 35, 45, 55, and 60 minutes of immersion [16]. The swelling ratio was calculated using Eq 1.

$$Swelling\ ratio\ (\%) = \frac{ds-dd}{dd} x\ 100\ \% \ldots \text{(Eq. 1)}$$

Where:

ds = diameter of the hydrogel film that has been soaked in distilled water.

dd = initial diameter.

2.8. In vitro Extract Release Test

Before conducting the extract release test, the maximum wavelength of the madeira vine leaves extract was determined using UV-VIS spectrophotometry. A 50ppm solution of the extract was scanned at wavelength of 400-800 nm [17]. The extract release test assesses the hydrogel's ability to release the extract, measuring the speed of release through percentage calculation of the mass of extract released into a liquid. The hydrogel film is placed in 20 mL of distilled water. As much as 5 mL of samples were pipetted every 30 minutes for three hours, and the same amount of fresh distilled water were added to replace the water taken. The concentration of the released extract was determined using UV-VIS spectrophotometry. The percentage of active substance released was calculated using a specific formula. The percentage of active substance released is determined using Eq 2.

$$Drug\ Release\ (\%) = \frac{r}{t}\ x\ 100\ \% \ldots \text{(Eq. 2)}$$

Where:

r = active substance release at time t

t = total active substance loaded in the film

3. Results and Discussions

3.1. Extraction

The extraction of madeira vine leaves produced 65.76 g pasta from 1900 g madeira vine leaves sample which correspond to 3.19 % yield. This result is lower compared to result described by Betriksia et al., (2018) who obtained 56.70 g from 500 g madeira vine leaves sample which correspond to 6.3% yield [12]. Aloe vera extraction produced 244.21 mL liquid from 500 g aloe vera gel sample which correspond to 16.26% yield. This result is higher compared to result described by Kurnia (2021), who obtained 90.27 mL liquid from 8000 g aloe vera gel sample which correspond to 1.125% yield [18].

The variation in extraction results is attributed to differences in solvent volume, as indicated by Nurussayyidah et al., (2018) who found that a larger material-to-solvent ratio leads to increased yield [19]. The choice of 96% ethanol as the solvent is based on its universal nature, and its polarity facilitates the attraction of desired compounds during extraction [20]. Research by Puspitaningtyas et al., (2021) reveals that higher ethanol concentrations result in higher yields [21]. Time plays a crucial role in the maceration method, as emphasized by Budiyanto (2008) [22]. Longer maceration times allow for extended contact between the solvent and active ingredients, while too short a duration may result in incomplete extraction. Temperature is another vital factor, where higher temperatures enhancing the solubility of extracted substances. However, caution must be exercised to prevent damage to the material, as excessively high temperatures can be detrimental [23-24].

3.2. Phytochemical screening results

Phytochemical screening in this study was carried out to identify phenolic, tannins, flavonoids, alkaloids, saponins, steroids and triterpenoids. The test results can be seen in Table 2. The phytochemical testing of madeira vine leaves extract revealed positive results for phenolic, tannin, flavonoid, saponin, and triterpenoid. This result is in agreement with result published by Indarto et al., (2019), except for triterpenoid which was not detected in their study [25]. Aloe vera extract showed positive results for flavonoid, alkaloid, and triterpenoid. This result is slightly different with work of Raphael (2012), who found that other than the previously mentioned compounds, aloe vera extract also contains tannin [26]. The variation in results among studies can be attributed to factors such as the type of solvent used and differences in the plant's growth environment [27]. Both madeira vine leaves and aloe vera extracts contain active compounds, including phenolics, tannins, flavonoids, and saponins, which are known for their potential antibacterial functions with different mechanisms of action [28].

3.3. Antibacterial Activity of Extracts

The antibacterial activity of the madeira vine leaves and aloe vera gel extracts are shown in Table 3. The assay was based on measuring the growth rate (μ) of *S. aureus* culture in the presence of 1000 ppm gentamicin as positive control, madeira vine leaves and aloe vera extracts. Culture with addiction of 1.5 mL 96% ethanol in 15 mL was used for negative control. The result of antibacterial activity showed that gentamicin has the highest bacterial growth inhibition as indicated of the lowest μ value (68.1% inhibition). Madeira vine leaves extract showed a high antibacterial activity, about half of gentamicin (39.13% inhibition). In contrast aloe vera extract did not showed any inhibition on bacterial growth as indicated by similar μ with negative control. The positive result of madeira vine leaves extract is in line with previous report by Mengga et al., (2022) and Wardani (2018), who reported high antibacterial activity of the extract [29-30]. This result indicate that madeira vine leaves extract is potential as antibacterial agent and can be applied in hydrogel. Even though aloe vera extract did not showed any antibacterial activity, it was still applied in the hydrogel preparation due to its moistening and cell-stimulating effects as reported by Dadashzadeh et al., (2020) [11].

3.4. Antibacterial Assay of Hydrogel

The results of the antibacterial activity of hydrogel with impregnated aloe vera and madeira vine leaves extracts combination is presented on Table 4. The data was then analyzed using Minitab 17 and the ANOVA table as the result of the analysis is presented in Table 5. Table 5 indicates that madeira vine leaves extract is the most significant factor to gives the highest antibacterial activity as indicated by the lowest P-Value (P= 0.051). While aloe vera extract, does not give significant effect on antibacterial activity (P= 0.471). This result is in agreement with the previously described antibacterial activity of extracts which showed that madeira vine leaves extract has antibacterial activity while aloe vera does not showed any inhibitory effect. Main effect plot of each extract indicates that the antibacterial activity increases when madeira vine leaves extract composition decreases and aloe vera increase (Figure 1). The highest antibacterial activity (lowest μ value) was observed when 250 ppm madeira vine leaves extract and 750 ppm aloe vera extract was combine into the hydrogel (Figure 2) [31]. This result indicates that there is a possibility of antagonist effect of aloe vera on antibacterial activity of madeira vine leaves extract, since increasing aloe vera extract reduce antibacterial activity of madeira vine leaves extract. From the tests conducted, it can be concluded that the best extracts combination impregnated on PVA/gelatin hydrogel is 750 ppm aloe vera and 250 ppm madeira vine leaves extracts. This PVA/gelatin hydrogel with this combination was further characterized for its chemical functional group, swelling ratio, and in vitro extract release.

3.5. FTIR (Fourier Transform Infrared) Analysis

FTIR characterization is carried out to determine the chemical functional group present in the hydrogel. FTIR characterization uses infrared spectroscopy in the wave number range 4000-400 cm^{-1}. The peak of FTIR spectrum at 3600-3200 cm^{-1} indicates the presence of -OH stretching which most likely come from hydroxyl groups in PVA, gelatin, glycerol and phenolic compounds from madeira vine leaves and aloe vera extracts. The peak spectrum at 3200-2800 cm^{-1} indicates the presence of C-H stretching which come from C-H in organic compound either in PVA, gelatin, glycerol, and phenolic compounds of the madeira vine leaves extract. Aromatic C=C stretching is detected by the presence of peak at 2200-2100 cm^{-1} which attributed to aromatic compounds in extracts of madeira vine leaves and aloe vera. Furthermore, peak at 1750-1735 cm^{-1} indicate the presence of C=O stretching from acetyl group especially gelatin. Peaks at 1583, 1575, and 1569 cm^{-1} were associated with peptide bond in gelatin (Figure 3).

3.6. Swelling Ratio

The result of the swelling ratio test is shown in Figure 4. The swelling test of the optimum hydrogel revealed a gradual increase in diameter over time, reaching 19.1% at the 60^{th} minute. The hydrogel, composed of gelatin, madeira vine leaves extract, and aloe vera, demonstrated its ability to expand, influenced by factors like polymer network (crosslinking), hydrophilicity, and concentration of polymer. Gelatin, acting as a binder, strengthens the PVA chain structure and enhances the hydrogel's hydrophilic properties, facilitating water absorption.

The study in agreement with previous research highlighting the benefits of combining polymers for improved water absorption [40]. The hydrogel-maintained consistency in swelling ratio over time, showcasing the elastic tension in the polymer chain network. The final yield percentage exceeded the standard specification of a swelling ratio reaching 12.5% within one hour, indicating the hydrogel's effectiveness in retaining its structure and swelling capacity [41].

3.7. In vitro Extract Release

Prior to *in vitro* extract release testing, it is necessary to determine the wavelength that can detect extract that were impregnated to the hydrogel.

The visible spectrum of madeira vine leaves extract is presented in Figure 5. There are two peaks detected in this test which are at 410 and 665 nm. For the *in vitro* extract release, the 665 nm wavelength was selected due to the moderate absorbance value at low concentration (50 ppm). The maximum wavelength was then used to measure the release of extract. The results of *in vitro* extract release are presented on Figure 6. The extract was released gradually, starting from 14.1% at the 30th minute, then slowly increased until it reached 28.5% in the 180th minute. This result indicate that the extract released in controlled manner, therefore suitable for the application as wound dressing [42].

Table 1: Experimental Design of Hydrogel Film Preparation.

Run number	Aloe vera extract concentration (ppm)	Madeira vine leaves extract concentration (ppm)
1.	250	250
2.	750	750
3.	250	750
4.	750	250
5.	250	250
6.	750	750
7.	250	750
8.	750	250

Table 2: Phytochemical screening results of madeira vine leaves and aloe vera extracts.

Compound Group	Madeira vine leaves extract		Aloe vera extract	
	This study	[25]	This study	[26]
Phenolic	+	+	-	-
Tannins	+	+	-	+
Flavonoids	+	+	+	+
Alkaloids	-	-	+	+
Saponins	+	+	-	-
Steroids	-	-	-	-
Triterpenoids	+	-	+	+

Table 3: Antibacterial test results of madeira vine leaves and aloe vera gel extracts.

Sample	μ (AU/hour)
Control (+)	0.044
Control (-)	0.138
Madeira vine leaves extract	0.084
Aloe vera extract	0.145

Table 4: Antibacterial assay results of hydrogels impregnated with combination of aloe vera and madeira vine leaves extracts.

Hydrogel Sample	Aloe vera extract concentration (ppm)	Madeira vine leaves extract concentration (ppm)	μ (AU/hour)
P1	250	250	0.033
	750	750	0.049
	250	750	0.027
	750	250	0.011
P2	250	250	0.004
	750	750	0.025
	250	750	0.032
	750	250	-0.015
Control (+)			-0.006

Note:
P1 = 1st replicate.
P2 = 2nd replicate.

Table 5: ANNOVA result.

Factorial Regression: μ (OD/hour) versus Blocks, Aloe Vera, Madeira vine leaves

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	0.002411	0.000603	4.83	0.113
Blocks	1	0.000684	0.000684	5.48	0.101
Linear	2	0.001335	0.000667	5.35	0.103
Aloe Vera	1	0.000084	0.000084	0.68	0.471
Madeira vine leaves	1	0.001250	0.001250	10.01	0.051
2-Way Interactions	1	0.000392	0.000392	3.14	0.175
Aloe Vera*Madeira vine	1	0.000392	0.000392	3.14	0.175
Error	3	0.000375	0.000125		
Total	7	0.002786			

Table 6: FTIR wavenumber values.

Function Group	Reference wavenumber (cm^{-1})	Wavenumber (cm^{-1})				Reference
		Control	Aloe vera	Madeira vine	A3B1	
-OH stretching C–H stretching	3600-3200 3200-2800	3073.37	3285.46	2882.98	3030.73	[32-35]
C=C stretching aromatic	2200-2100	2159.11	2163.20	2163.41	2163.32	[36]
C=O stretching	1740-1720	1725	1738	1734	1731	[37]
C-N stretch dan N-H bending	1600-1400	1569	1426.28	1583.03	1575.65	[38-39]

Note:
A3B1 = Optimum hydrogel impregnated with 750 ppm aloe vera and 250 ppm madeira vine leaves extracts.

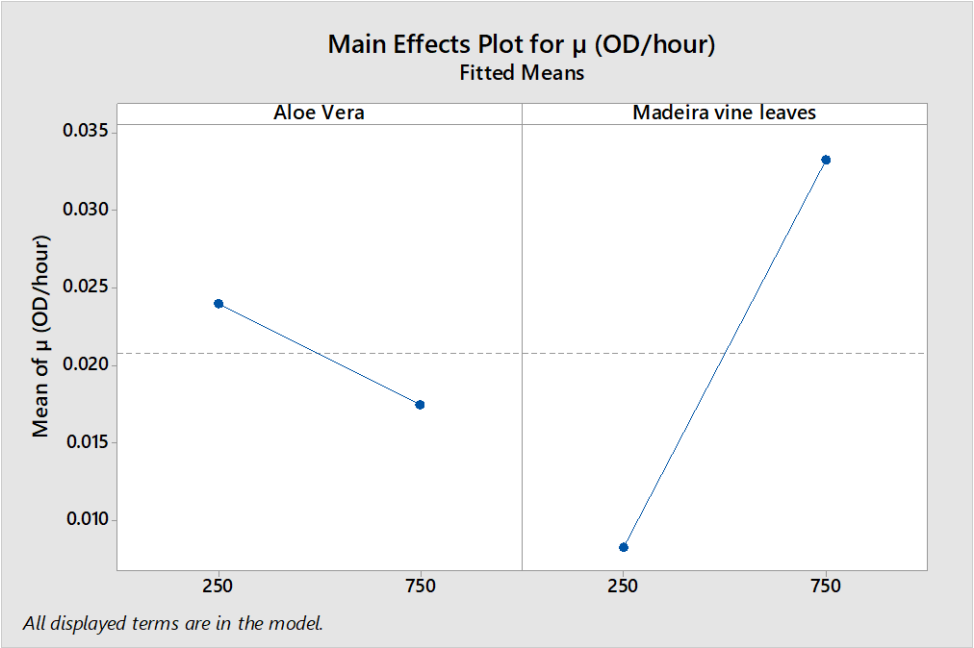

Figure 1. Main effects plot.

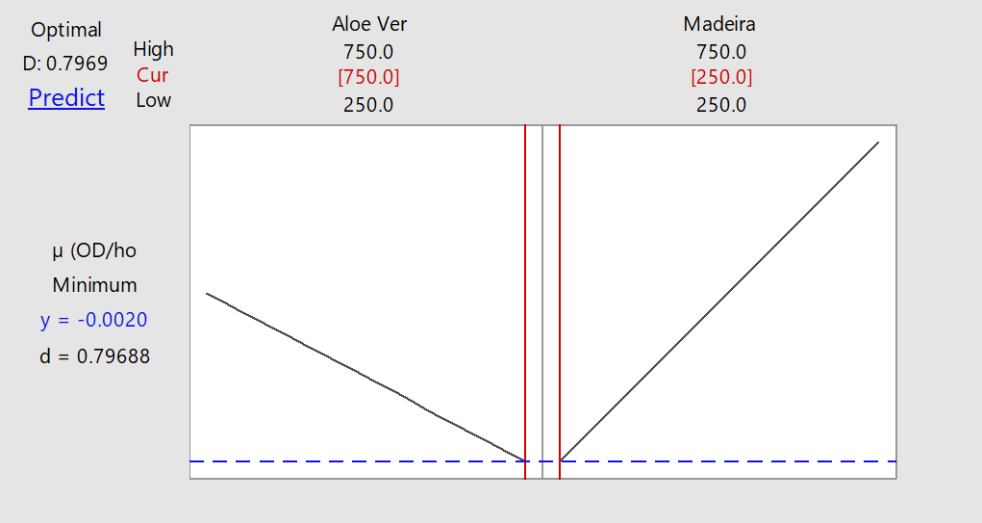

Figure 2. Response optimization.

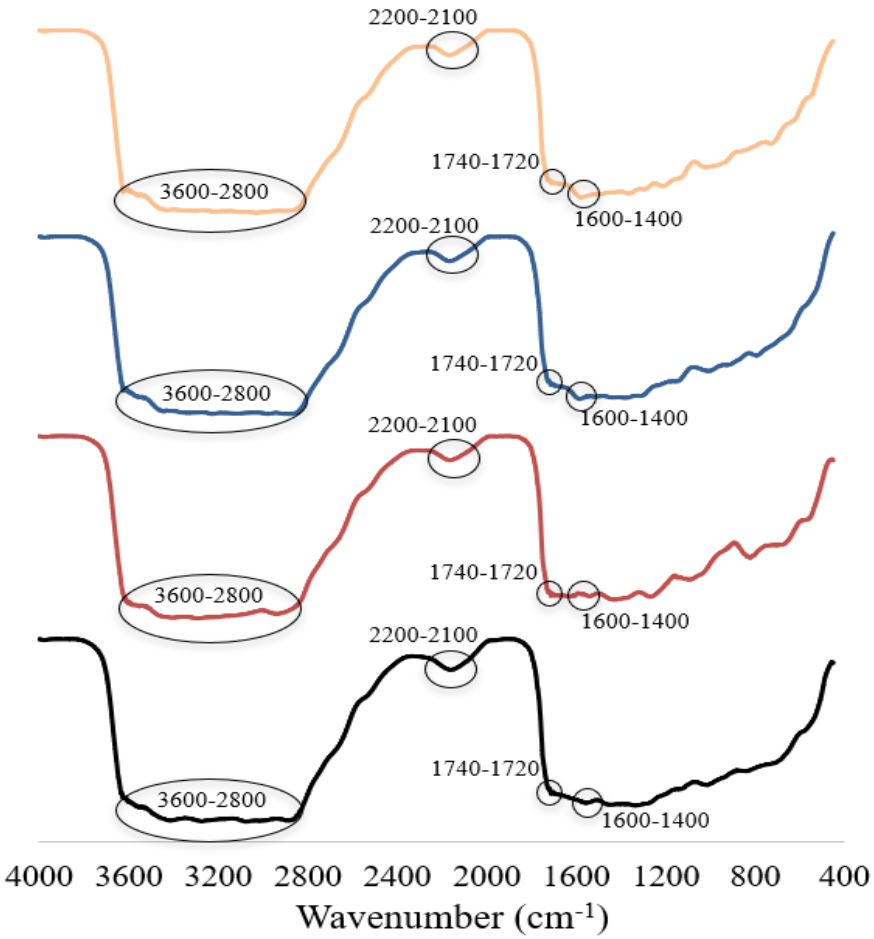

Figure 3. FTIR spectrum of PVA/gelatin hydrogel (black), PVA/gelatin hydrogel impregnated with aloe vera (red), madeira vine leaves (blue), and the optimum hydrogel (orange).

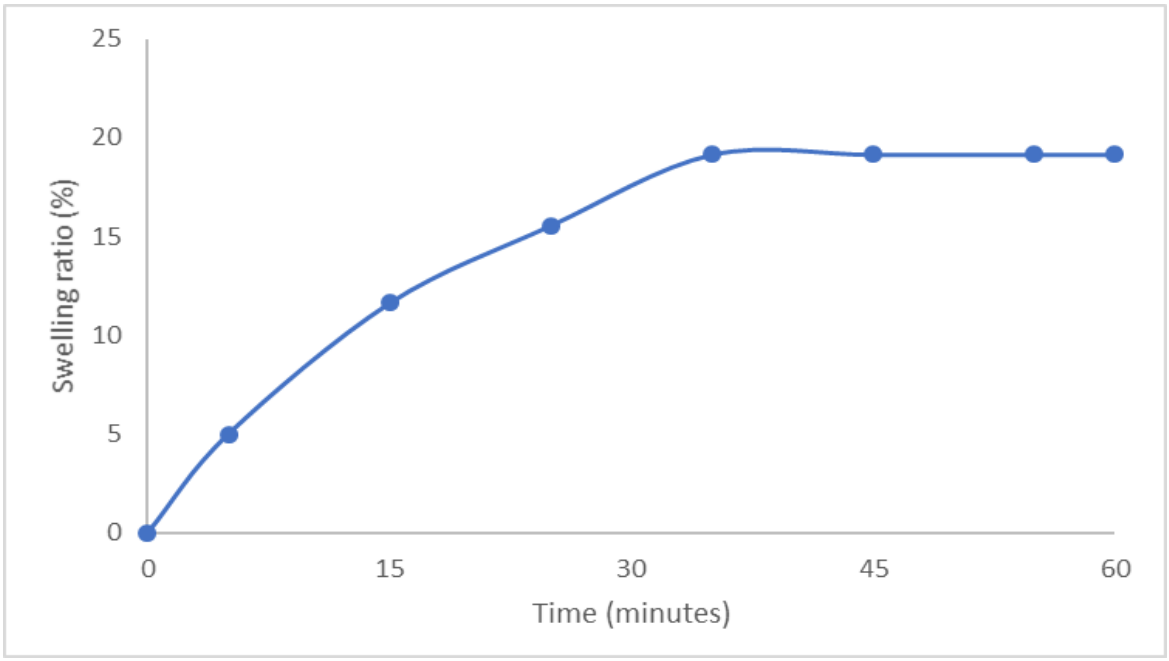

Figure 4. The results of the swelling ratio of the PVA/gelatin hydrogel impregnated with 750 ppm aloe vera and 250 ppm madeira vine leaves extracts.

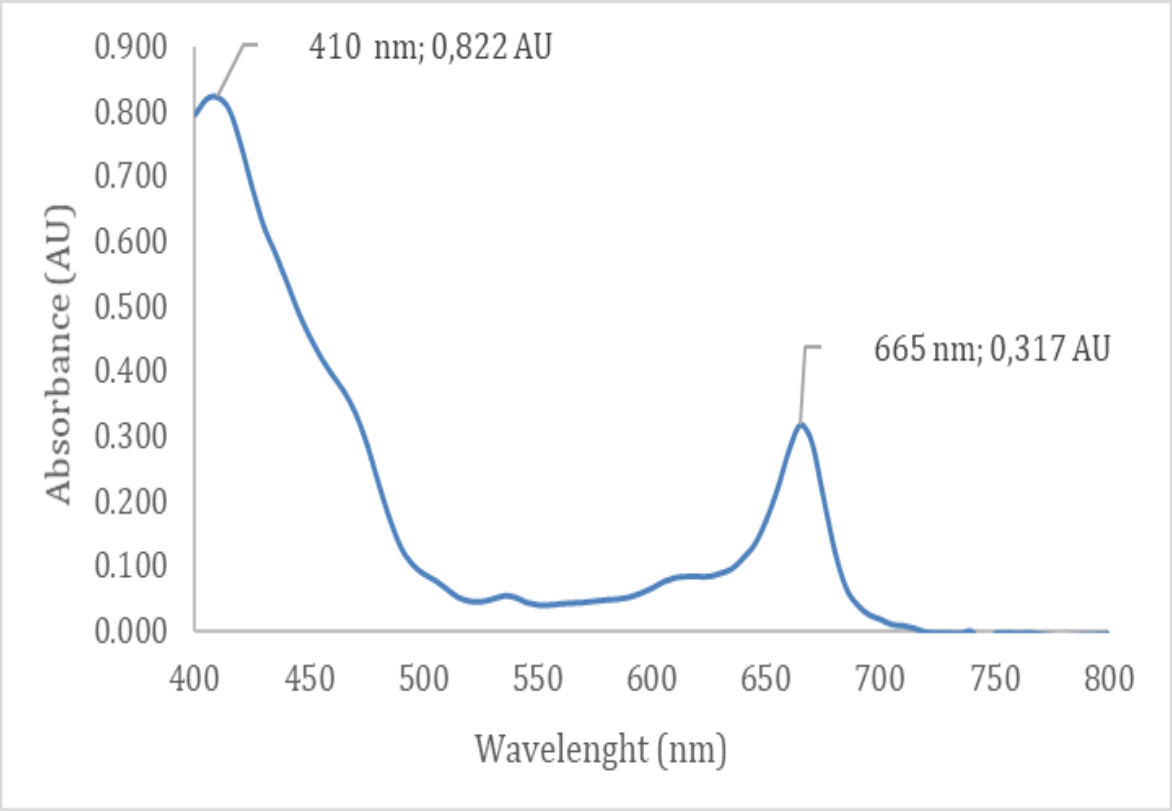

Figure 5. Visible spectrum of 50 ppm madeira vine leaves extract.

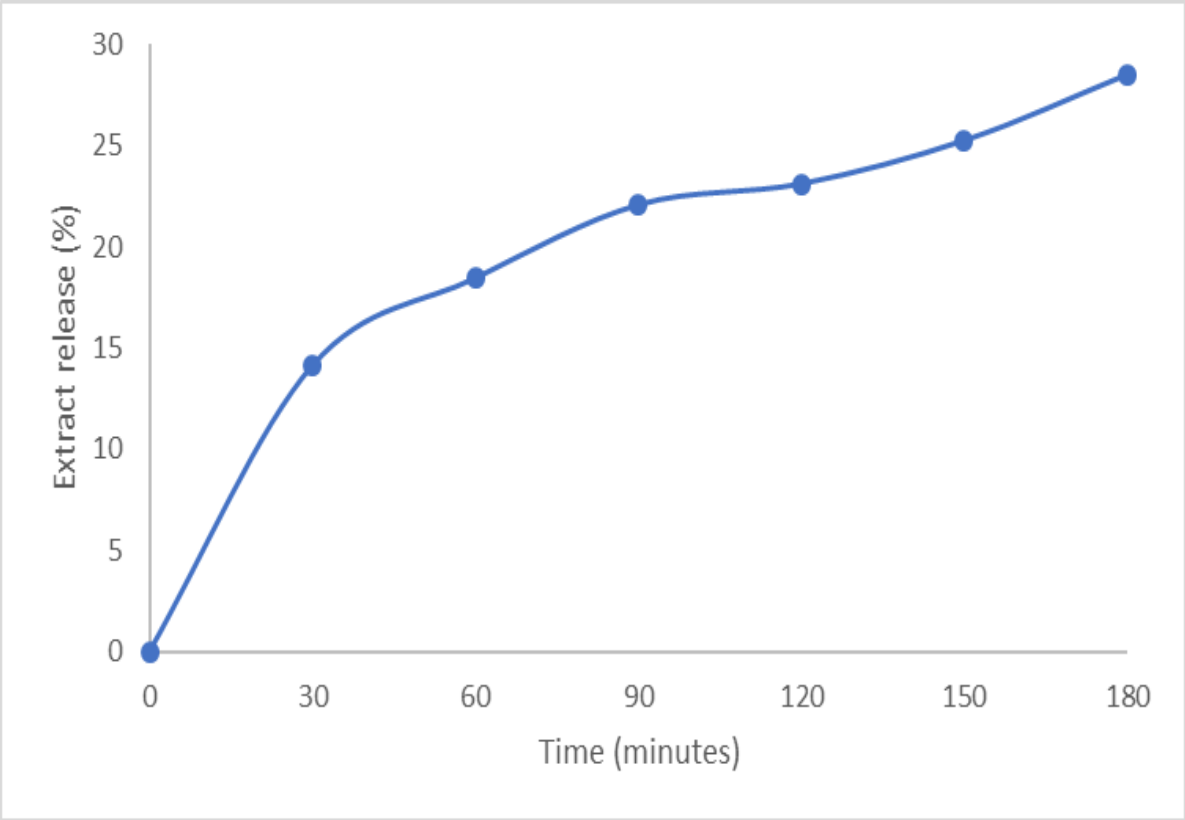

Figure 6. *In vitro* extract release at 665 nm from PVA/gelatin hydrogel.

4. Conclusion

Based on the results of the present study, the highest bacterial activity was found at 250 ppm madeira vine leaves extract and 750 aloe vera extract combination in PVA/gelatin hydrogel. FTIR analysis confirm the presence of -OH stretching, C-H stretching, aromatic C=C stretching, C=O stretching, and peptide bond. The swelling ratio of the hydrogel is 19.1% which exceeds the standard specification of the swelling ratio of 12.5%, and the *in vitro* extract release indicate that the 28.5% extract was release in 180 minutes. The data collected confirm that the PVA/gelatin hydrogel impregnated with madeira vine leaves and aloe vera extracts is suitable for wound dressing.

Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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